

P.K

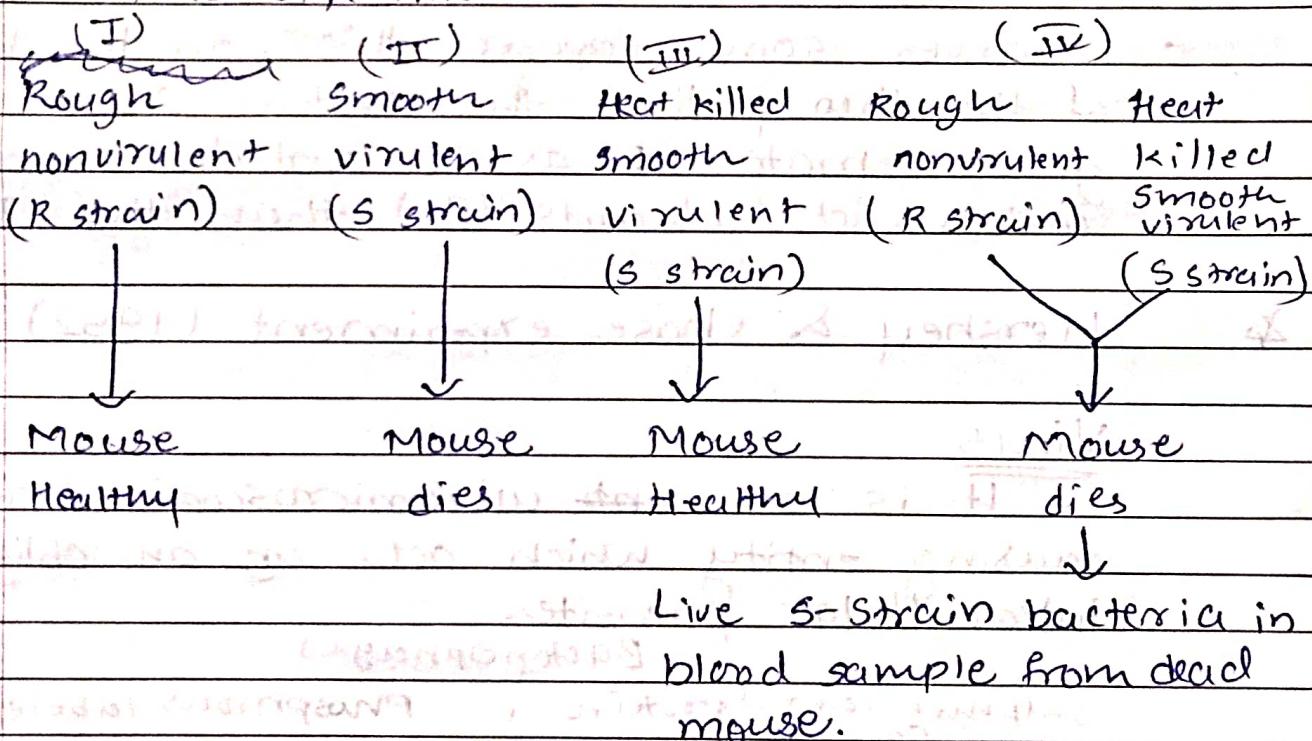
30.01.2021

GENETIC ENGINEERING

- Cells are made up of biomolecules, which are very essential for the metabolism
- Biomolecules arranges themselves & cells are formed.
- Biomolecules
 - ↳ carbohydrates
 - ↳ proteins
 - ↳ lipids
 - ↳ nucleic acids



Griffith's Experiment.



- Transforming Principle:



Oswald Avery, Colin MacLeod & Maclyn McCarty

- Experimented in 1944.
- Recovered all the biomolecules from the ~~R-strain bacteria present in~~ ~~blood sample of~~ S-strain dead mouse.

- Biomolecules from dead mouse & R strain

→ carbohydrates + R strain = R strain
 → proteins + R strain = R strain
 → lipids + R strain = R strain
 → nucleic acid + R strain = S strain

- Which nucleic acid

→ Nucleic acids + R strain + DNase = R strain
 → Nucleic acid + R strain + RNase = S strain.

- From this experiment, it is proved that the transforming principle in Griffith's experiment is DNA.
- However, some opposed this, on the basis of the theory that the protein should be genetic material as it contains more amino acids/subunits (21) than the DNA (4).

* Hershey & Chase experiment (1952)

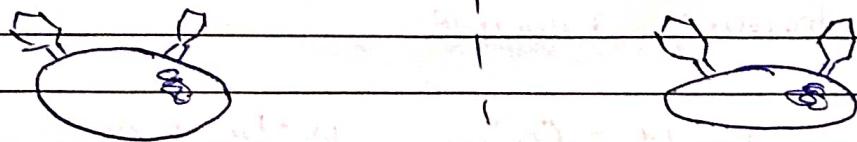
Virus

It is an ~~unt~~ ultramicroscopic disease causing entity which acts as an obligatory intracellular parasite.

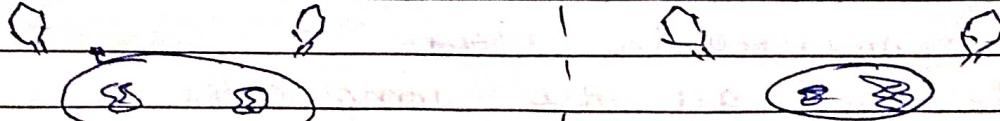
Bacteriophages

sulphur labeled protein, Phosphorus labeled DNA

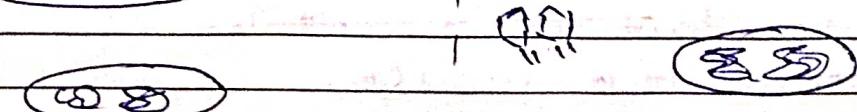
Infection



Blending



centrifugation



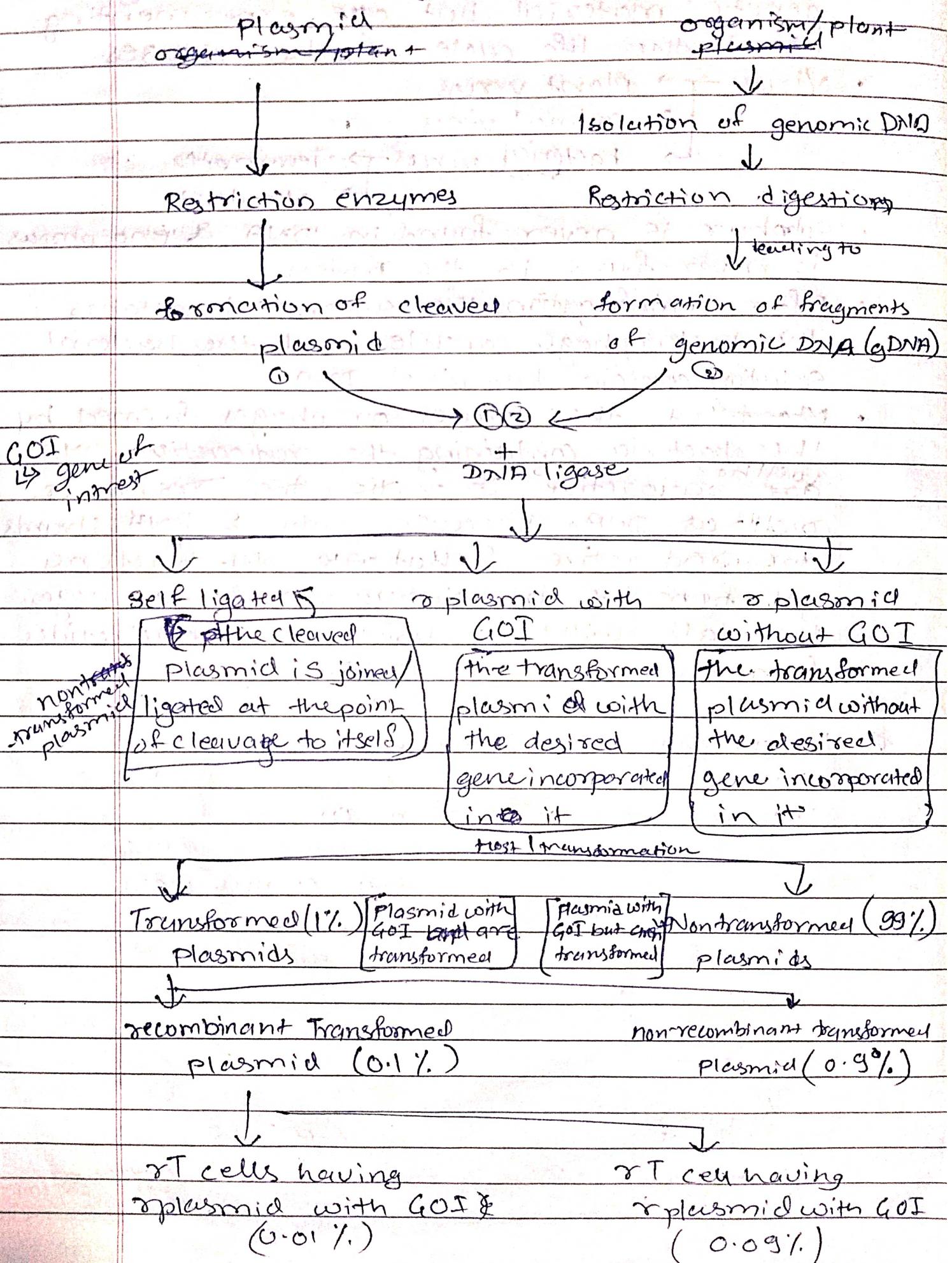
Supernatant showed radioactivity

Bacterial solution showed radioactivity

- Hershey & Chase weren't discovering the genetic material but were experimenting about the life cycle of the viruses.
- Virus → plant virus
→ animal virus
↳ bacterial virus (bacteriophage) → Temperate → Virulent
- Sulphur is never found in DNA & phosphorous is never found in the protein.
- After centrifugation, the supernatant contains the bacteriophage capsule and the bacterial solution contains the viral DNA.
- ~~Most~~ 4 of the viruses or phages formed by the bacteria containing the radioactive DNA will have radioactive capsular DNA. Because only 2 DNA strands are radioactive & that ~~one~~ ~~the~~ bacteria got from the radioactive virus or phage. Rest all will be using the nucleotides from the bacteria itself.

Agarose Gel Electrophoresis

Agarose Gel Electrophoresis



- Transformation
 - ↳ Exchange / Transfer of exogenous DNA into the host cell extracellularly.
 - ↳ 2 diff. types
 - Natural
 - Artificial
 - ↳ CaCl_2 (Prokaryotes)
 - ↳ Electroporation
- A variety of enzymes are used to get the αT cells having α -plasmid with GOT in the large number or ~~ext~~ greater % as only 0.01% of those are formed.

* Nucleases

- Enzymes that depolymerises nucleic acids.
- DNase
 - Enzyme that depolymerises the DNA
- RNase
 - Enzyme that depolymerises the RNA
- DNase
 - Exonuclease → cleaves & completely degrades DNA
 - Endonuclease → cleave internal phosphodiester bonds of double stranded DNA.
 - doesn't act on circular DNA.
 - Exonuclease → cleaves the DNA from the ends
 - Completely degrades DNA to dNTPs (free).
 - acts on circular as well as linear DNA.
- In prokaryotes, there's restriction ^{systems} enzyme consisting of restriction enzymes that acts as immune system by cleaving or degrading viral genome.

→ Restriction Systems

- In prokaryotes, it contains the various restriction enzymes.
- As soon as the viral DNA enters prokaryotic cytoplasm, the Restriction enzymes mainly endonucleases acts on viral DNA & degrades it, as Restriction System comes to know that the newly introduced viral DNA is foreign and not self.

• Restriction enzymes are specific to ^{certain} DNA sequences.

→ Modification Systems of self & foreign.

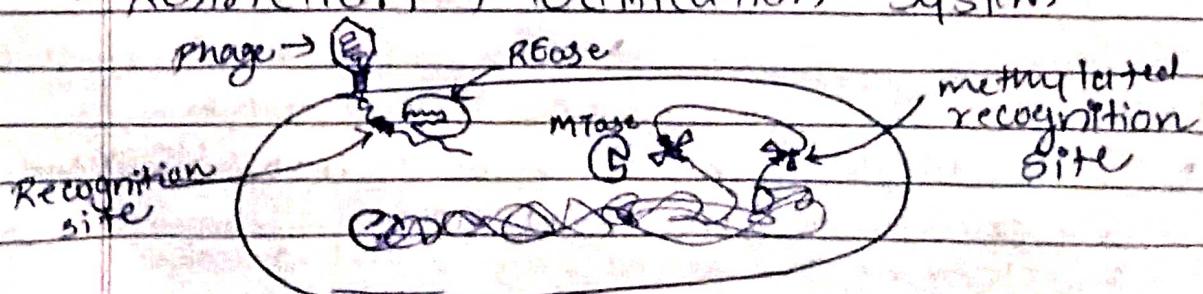
- To protect the bacterial DNA, prokaryotes have modification system.

→ Modification System:

- Enzyme such as Methyl transferase & glycosyl transferase are used to modify the one or both the strands of ~~DNA~~ bacterial DNA.
- Methyl transferase is most common & glycosyl transferase is most rare.
- This system protects the bacterial DNA from getting degraded ~~from~~ by action of Restriction System.
- Sometimes, the viral DNA also gets methylated. During that times the viral DNA survives and the bacteria gets infected.

→ RMS

- Restriction Modification System



P.K
30.01.2021

GENETIC ENGINEERING

Wrote 8 = Summary (Ref. Colgate)

1. Lehninger

2. + Genetics

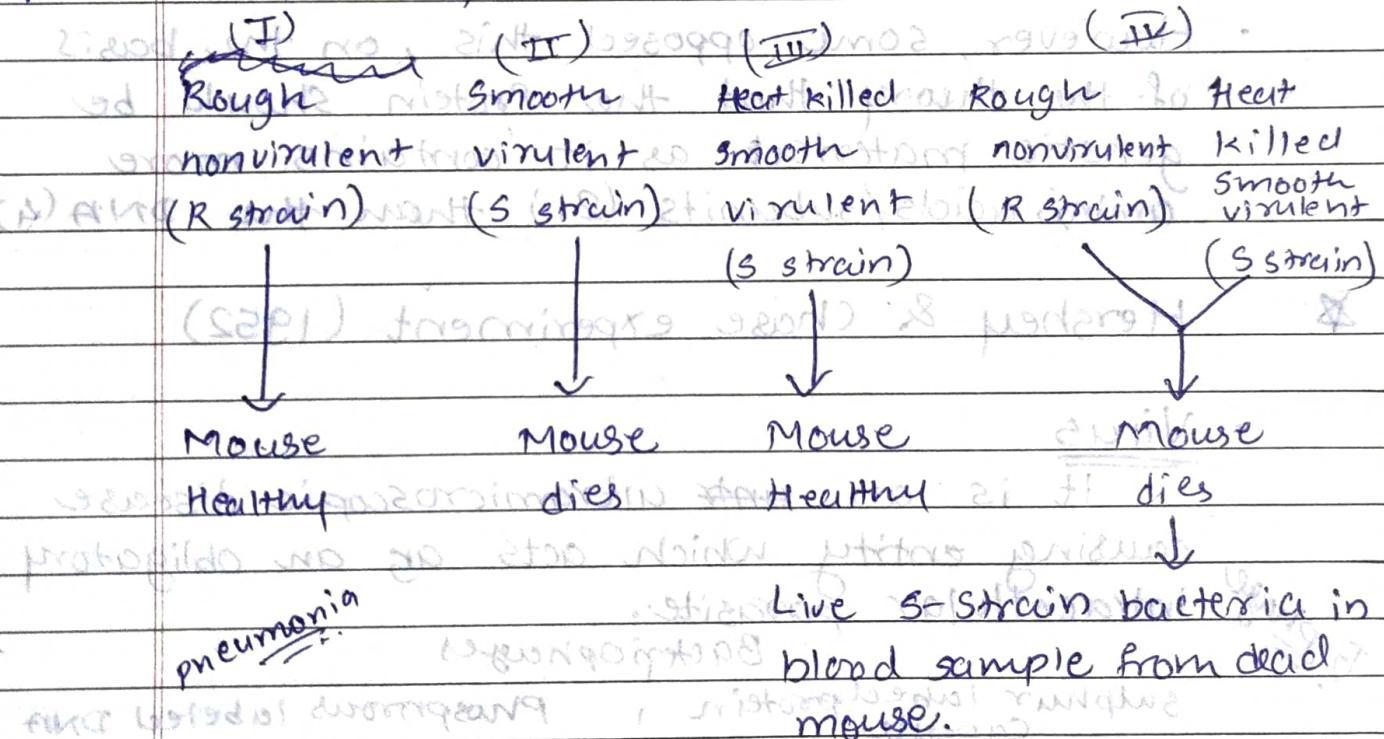
3. principles of gene manipulation

- Cells are made up of biomolecules, which are very essential for the metabolism
- Biomolecules arrange themselves & cells are formed.
- Biomolecules
 - Carbohydrates
 - proteins
 - Lipids
 - Nucleic acids

with front

transferring antigenic properties

* Griffith's Experiment:



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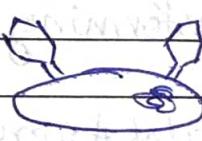
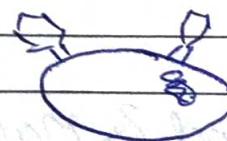
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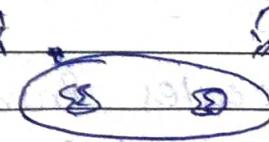
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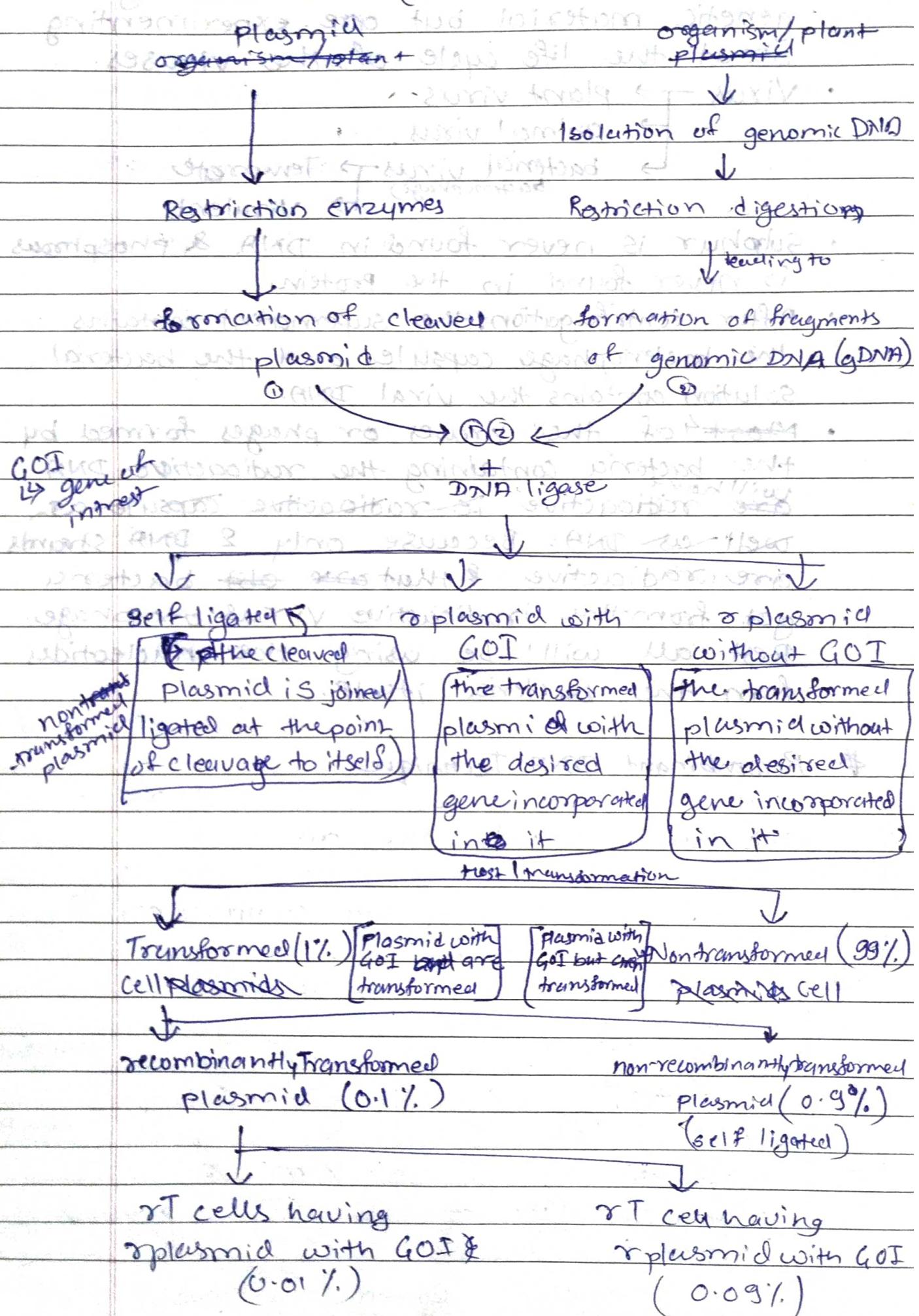
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Recombinant DNA Technique

~~Agarose gel~~ ~~Agar~~ r-DNA Technique: ~~genetic~~ & ~~genetic~~



- Transformation

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↳ Artificial

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↳ Electroporation

A variety of enzymes are used to target the λ T cells having rplasmid with GAT in the large number or ~~not~~ in greater % as only 0.01% of those are formed.



Nucleases - cause apoptosis avoided

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→ DNase

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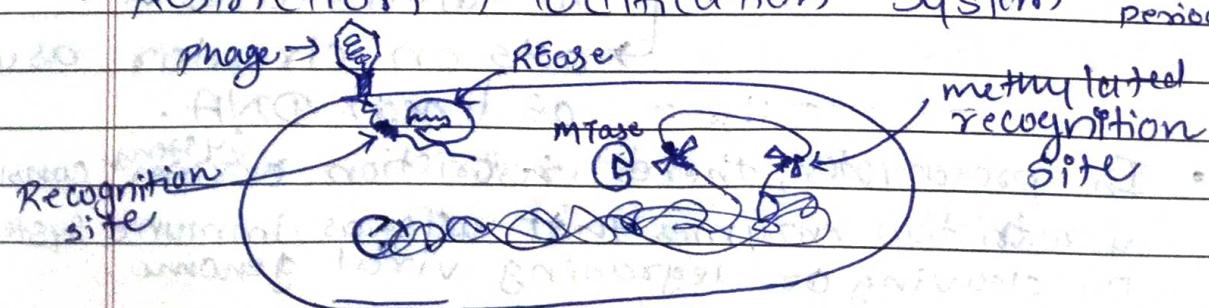
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- Methyl transferase is most common & glucosyl transferase is most rare.
- This system protects the bacterial DNA from getting degraded ~~by~~ by action of Restriction System.
- Sometimes, the viral DNA also gets methylated. During that times the viral DNA survives and the bacteria gets infected.
- If DNA gets hemimethylated i.e. only one strand is methylated then the other one will also get methylated after some period of time.

- **RMS** → then the other one will also get methylated
- **Restriction Modification System**



- * **Restriction Enzymes:**
- Type I ⇒ (same as type III)
 - Type II ⇒
 - Type III ⇒ specific at ~~the~~ its own restriction site but can't cleave within the restriction sites. cleaves anywhere.

→ **Type II R.E.:**

- appears as single polypeptide chain
- 20-100 KDa (wt)
- Mg^{+2} for activation, unlike type II & III that needs ATP
- extremely specific towards the restriction site.
- cleaves DNA within the recognition sequence
- gives either blunt or sticky ends.

* **Characteristics:**

Nomenclature: 1st letter ⇒ genera of bacteria

- next 2-3 letters ⇒ species of bacteria
- next letter ⇒ strain of bacteria
- Roman numeral ⇒ order of discovery
- Eg. Eco RI , Bam HI , Hind III

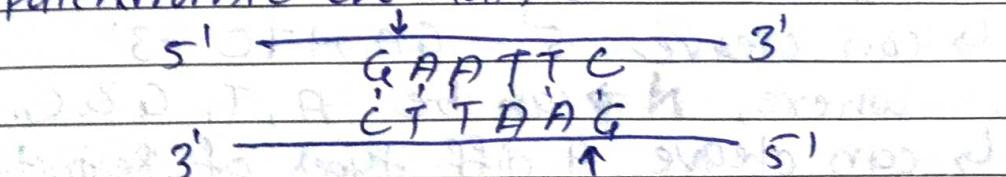
E. coli R-strain I

B. amylo liquefaciens H-strain I

H. influenzae D-strain III

→ **Restriction site / Recognition sequence:**

- mostly ~~based~~ made up of 4, 6 or 8 base pairs of nucleotides.
- two fold axis of rotational symmetry.
- palindromic in nature.



\rightarrow Frequency of occurrence of nucleotide

(more is seen)

- The frequency at which a particular nucleotide gets repeated in the DNA strand.
- It can be calculated as

$$\frac{1}{4^n}$$

where $n \Rightarrow$ no. of base pairs found in given restriction site

- Base pairs

e.g. ATGC → 4 base pairs

ACCG → 4 base pairs

∴ Its frequency is

Chances of finding ATGC = $1 / 4^4 = 1 / 256$ = 0.0039

ux⁴ The sequence repeats itself after $16 \times 4 = 256$

" 64 × 4 = 256

256 = 2⁸ → 8 base pairs

\rightarrow Classification of type II RE.

- frequent \Rightarrow 4 or 6 base pairs

cutter I cutter II ends up with

III cutter II ends up with

- Rare \Rightarrow 8 base pairs

cutter, no cleavage at other positions

\rightarrow Degenerative RS (Restriction sites)

- Grindam & Sanger method
- Strenuous ligation in DNA blot analysis

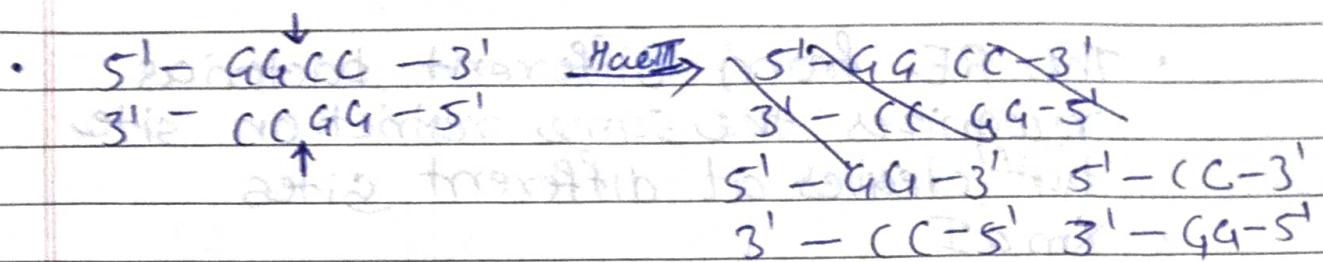
HinfI

↳ can cleave $5' - \text{GANTC} - 3'$

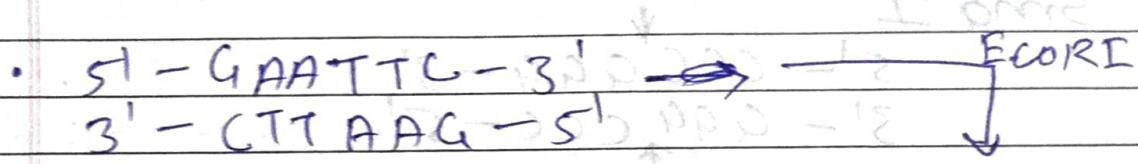
where, N \Rightarrow can be A, T, G & C.

↳ can cleave 4 diff. types of restriction sites.

→ Mode of action



- Cleaving is happened at the same point on both the DNA strands.
- This produces blunt ends.



- Cleaving is happened at the different points on the two DNA strands.
- This produces sticky ends.
- When cleaves near 3', 3' overhang is produced.
- When cleaves near 5', 5' overhang is produced.

→ Isoschizomerase reaction not mentioned

- pair of R.E. obtained from two different organism with different genera, different species, but recognise & cuts at the same site.
- MspI & KpnII

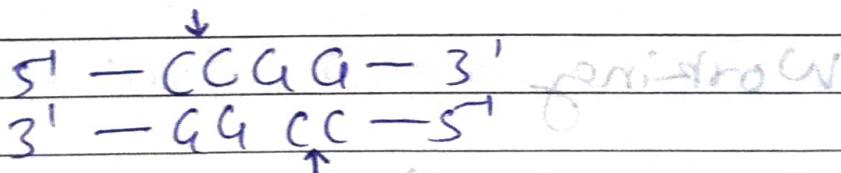


Diagram illustrating the mode of action of KpnII restriction endonuclease:

Initial state: $5' - \text{GGCC} - 3'$ (top strand) and $3' - \text{CCGG} - 5'$ (bottom strand).

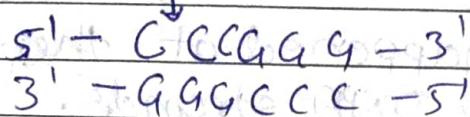
Cleavage: KpnII cleaves both strands at different sites, creating sticky ends.

Result: $5' - \text{GG} - 3'$ and $3' - \text{CC} - 5'$.

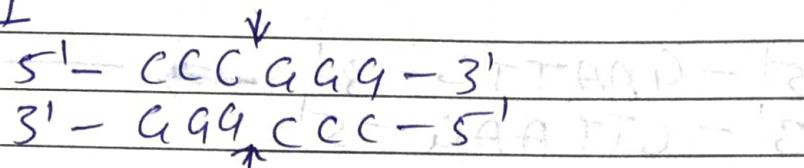
→ Neoschizomeric restriction endonucleases

- The R.E. from different bacteriaas recognizes the same restriction site but cleaves at different sites

Xba I



Sma I



→ Stereoselectivity of RE II

Reasons for Stereoselectivity

- use of non-ideal strength buffer
- excess or very less amount of DNA
- Excess of R.E.
- High glycerol concentration
- Ethanol, DMSO, phenol contamination
- Prolonged incubation time
- Non-optimum temperature

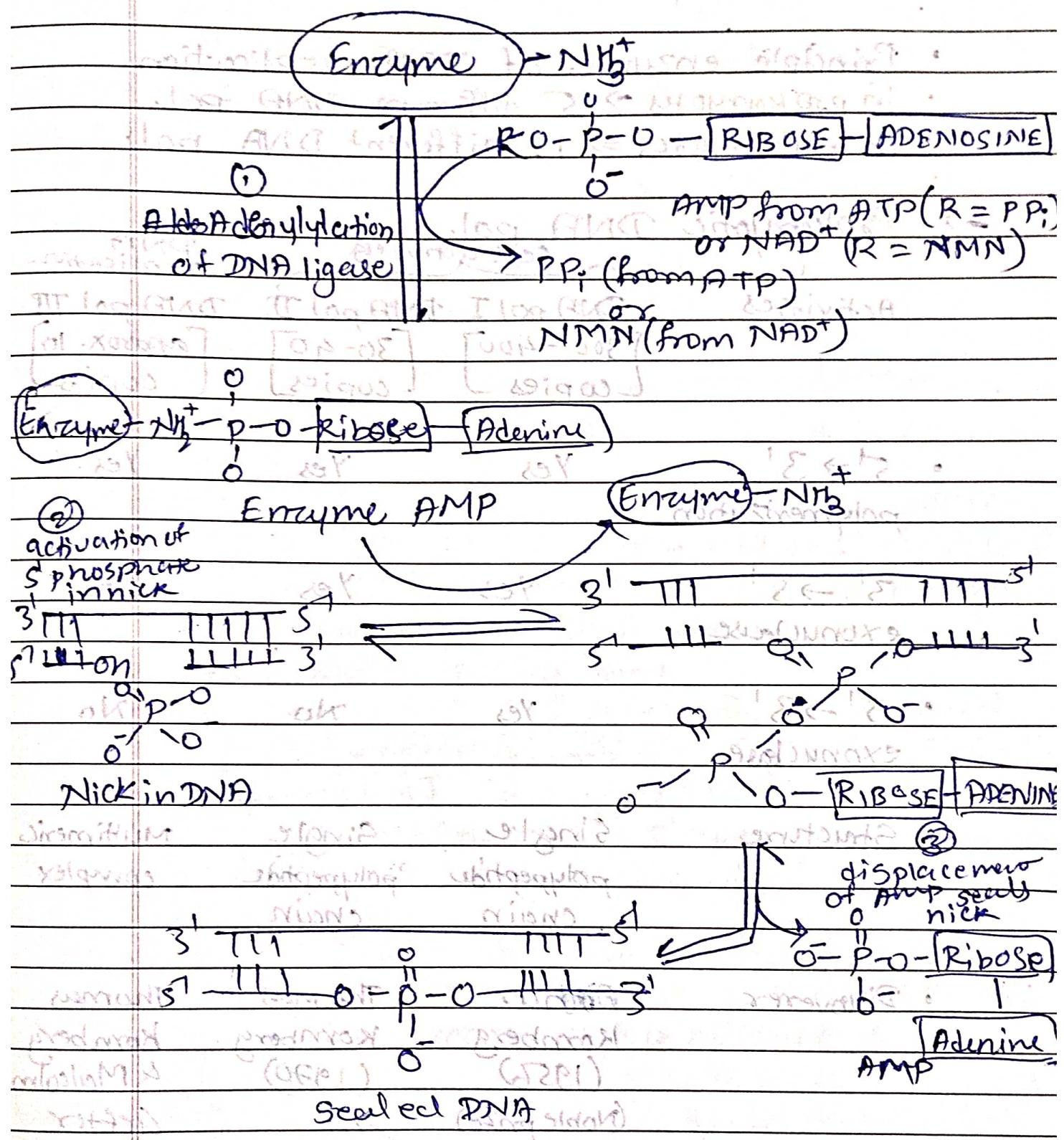
DNA Ligase:

- Molecular structures / Molecular glue
- DNA replication
- DNA repair

→ Working

- Sticky ends stick together but the gap remains
- This gap is recognized by Ligase
- Ligase sticks / seals the gaps by facilitating the phosphodiester bonds.

→ Mode of Action



→ Types of DNA Ligase

- E. coli DNA ligase $\Rightarrow \text{NAD}^+$ (cofactor reqd to become active)
- T4 DNA ligase $\Rightarrow \text{ATP}$ (cofactor reqd to become active)